FORMICA INTEGRA (HYMENOPTERA: FORMICIDAE) IV. EXCHANGE OF FOOD AND TRICHOME SECRETIONS BETWEEN WORKER ANTS AND THE INQUILINE BEETLE, CREMASTOCHEILUS CASTANEUS (COLEOPTERA: SCARABAEIDAE)

by

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With 20 figures

ABSTRACT

Experimental evidence, using radioactive tracers, confirms the role of *Cremastocheilus castaneus* as a predator in the ant nests, as well as proves the existence of trichomes on the beetle which supply substances transmitted by ants through the social organization of the nest. A specific new ventral trichome area was discovered on the beetle, using this technique. The trichome areas are illustrated with scanning electron microscope photographs.

To conduct studies on biology, morphology, and foraging behavior of an ant, *Formica integra* Nylander, we transferred two nests of this forest ant from its natural habitat in Georgia (for description of locality, see Kloft et al., 1973) to the Forest Insect Research Laboratory, University of Florida, Gainesville, Florida in 1972. This ant, of the subfamily Formicinae, is the southernmost representative of the *Formica rufa* group in eastern United States. The studies about the possibilities of an introduction of this ant into Florida forests could be continued; two more papers are cited (Wilkinson et al., 1978, 1979, in press).

During our laboratory experiments, three specimens of a scarab beetle, *Cremastocheilus castaneus* Knoch, flew from the nests established in open arenas in a fully climatized laboratory (14 hrs light, 25 ± 2° C, 50—80% rel. air humidity). Possibly the change in environmental conditions between the natural colony and the laboratory induced emigration of the beetles.

The genus *Cremastocheilus* Knoch has long been in need of revision. The most recent general paper is that of Potts (1945), which provides a key to North American species. Because of the numerous short scattered setae on the pronotum and the geographic distribution, our specimens would key to *C. castaneus brevisetosus* Casey. Potts (1945: 74) stated: "Casey describes

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brevisetosus from a specimen he records from Iowa. However, the only specimens before me which agree are from Alabama, and I am led to suspect an erroneous label on the Casey specimen. In the Alabama specimens the setae of the pronotum are exceedingly broad and short and as the specimens are quite fresh, the character is presumably a good one. This appears to me to be the most distinctive race of any I have seen, and if this name is applicable to the southern specimens it apparently represents a valid subspecies. The value of the other two names as weak races of *castaneae* seems questionable to me. A long series from the Rocky Mountains is not easily separable, although in the main, they most closely agree with Casey's *pocularis*."
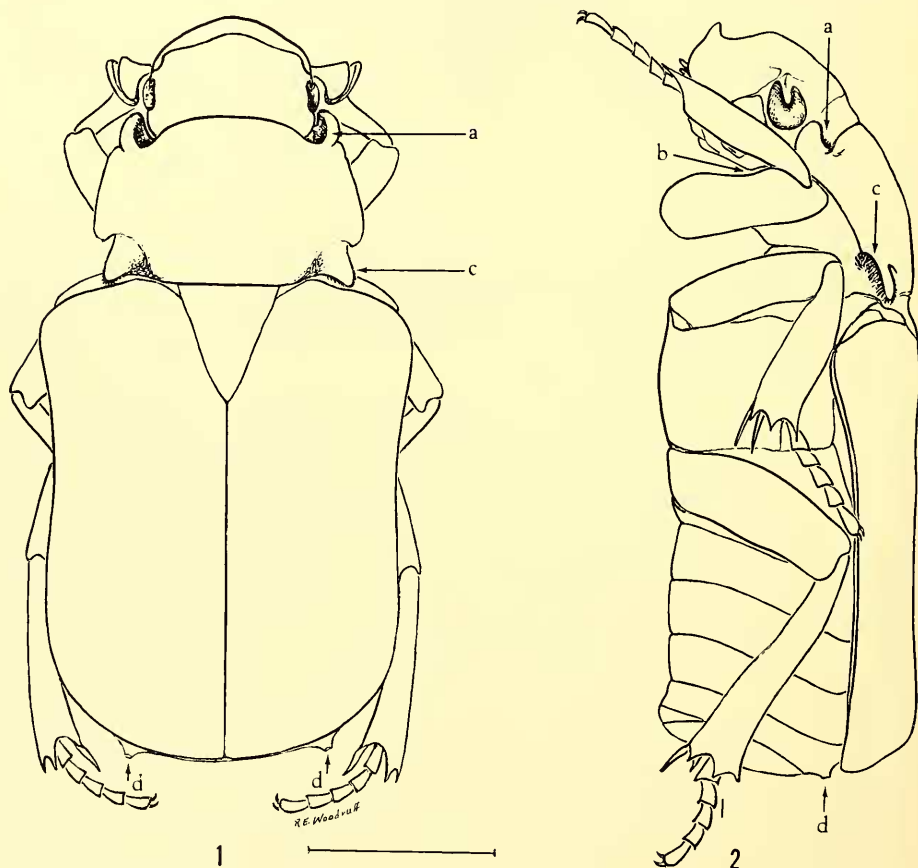
Figs. 1—2. Outline drawings of *Cremastocheilus castaneus brevisetosus*: 1) dorsal view; 2) lateral view: a) anterior pronotal trichome area; b) location of prosternal apophysis with trichomes; c) posterior pronotal trichome area; d) propygidial spiracle. (Scale line equals 2.5 mm).

The genus *Cremastocheilus*, which comprises more than 40 species, is endemic to North America. All known species live as adults in the nests of ants of 11 genera; *C. castaneus* has been recorded in association with *Formica* and *Polyergus* (Cazier & Statham, 1962). Wheeler (1910, 1928) reported it from *F. integra* nests. The first

monographic revision was given by Horn (1879); Casey (1915) described many novelties and revised the North American Cremastocheilini together with other groups of Cetoniinae. The species-level review by Potts (1945) has already been mentioned. Howden (1971) and Krikken (1976), proposing new genera, presented keys to the Western Hemisphere genera of Cremastocheilini.

Cazier & Statham (1962) pointed out that the true relationship between species of the genus *Cremastocheilus* and their host ants is not yet known. In observations of a western species *(C. stathamae* Cazier) in nests of the honey-ant, *Myrmecocystus mexicanus* Wesmael, Cazier & Mortenson (1965) found these beetles were obligate predators of the ant larvae. At the time this was the first recorded case of primarily predatory behavior within the family Scarabaeidae. W. M. Wheeler (1910) described the trichomes on the anterior and posterior prothoracic angles of the beetles and presented a figure of a *Formica integra* worker gnawing on one of the trichomes (see also this figure reproduced in Wilson (1971, fig. 20—4)). Alpert & Ritcher (1975) stated that *C. armatus* Walker adults were predaceous on both ant larvae and pupae, and that "Beetles were not disrupted from feeding even when covered with attacking ants."

Some of the observations of Cazier & Mortenson (1965) appeared very contradictory to them. As Wilson reported (1971: 390) the ants treated the beetles sometimes as synechtrans and sometimes as symphiles. Most of the time, however, they had the status of synoeketes; i.e., they were ignored and allowed to wander through the nest without interference.

Tracer studies on exchange of food and trichome secretions between ants and beetles

As shown by Goesswald & Kloft (1958, 1960, 1963) radioisotopes are a useful tool to elucidate interrelations within societies of social insects. Hoelldobler (1967, 1970) used ^{32}P to demonstrate the physiology of guest-host relations (myrmecophily) in ants. We used radioactive food (20% sucrose solution + $Na_2H^{32}PO_4$, specific activity 0.5 mCi/ml) to trace social food exchange in worker groups of *Formica integra*. All experiments were conducted in 1972, with dates shown.

EXPERIMENT I

7.vi: Worker ants were fed with ^{32}P honey and after 18 hrs checked under an end-window G. M. tube. The detector was connected with a Berthold-Frieseke Scaler-Timer System BF 22/25. Before checking, the insects were carefully decontaminated with a "chaser" solution plus detergent (Kloft, 1977). The ants had an average radioactivity of 20,000 CPM.

20.vi: At 12:00 noon one beetle (A) was put together with 10 radioactive-fed ants of *F. integra*; no food was added. At 3:45 PM the beetle was activity besieged by the ants which palpated it intensively with their antennae. Ants were sitting on and underneath the beetle's head, gnawing on the trichomes. Since we supposed food transfer from the ants to the beetle, we checked the beetle at 3:50 PM for radioactivity. The geometry was like that used for the measurements of the ants.

Result: After 3 hrs 50 min the beetle showed 3033 CPM. We checked at 5:30 PM and the activity was 3124 CPM or about the same. All ants were in good condition.

21.vi: At 9: 00 AM the beetle was in good condition, but all ants were dead, the gaster squeezed out. In some cases we found parts of the dead ants, presumably dismembered by the beetle.

Experiment II

22.vi: One beetle (B) was put with 10 radioactive-fed ants. The beetle displayed a death feint (Totstell-Verhalten), but was palpated by the ants. The palpation itself did not lead to contamination, as shown by subsequent checkings.

23.vi: After 22 hrs the beetle showed a total activity of 1600 CPM, and all 10 ants were in good condition. Presumably the beetle must have participated in the social distribution of radioactive-labelled food. Four hours later, after a total time of 26 hrs, the beetle had about the same rate of counts. However, 2 ants were found dead, and one of these appeared to be lacerated by the beetle.

In both experiments the ants were not only attracted to the trichomes on the anterior and posterior prothoracic angle of the beetles, but also soliciting, by antennal movements, certain parts of the sternum. These observations led to the detection, for the first time, of ventral hair tufts (trichomes) on the prosternal apophysis, described in the following part. As best we could observe, the beetle lowered the anterior portion of the mentum, which completely covers the mouth parts. The palpi could be seen moving in and out. We believe that by such movements the regurgitation by the ant is released. The food transfer could not be observed directly, but was evident by the increase of the beetle's radioactivity long before any ants were killed or squeezed out.

Experiment III

22.vi: Beetle (A) was carefully decontaminated and subsequently checked for radioactivity. At 11: 45 AM it had 20.810 CPM. Certain inherent problems are encountered in radio-isotope work. High counts are found when the radioactivity is broadly distributed. When the radioactivity is localized, lower counts result, even if the absolute radioactivity is the same. Equal distribution of the ^{32}P,

Table 1. Transfer of radioactivity from a radioactive labelled *Cremastocheilus castaneus* to worker ants of *F. integra* via the trichome secretions within 3 hrs.

	COUNTS	COUNTING DURATION (MIN)	CPM	$\pm$ SQ. RT. MIN
BACKGROUND	61	5	12.2	+ 3.49
BACKGROUND	43	5	8.6	+ 2.93
ANT A	155	5	31	+ 5.56
ANT B	121	5	24.2	+ 4.92

throughout the hemolymph, is probably responsible for this high count, although body absorption could have affected the counts (Kloft, 1962). This complete distribution within the body was a precondition for the following experiment: the radioactive-labelled beetle (A) was put together with 10 nonradioactive worker ants. The object was to determine if there were transfer of radioactivity, via trichome secretions, from the beetle to the ants.

22.vi: The ants had been around the trichomes gnawing on the hair tufts. At 2: 45 PM, after a total elapsed time of 3 hrs, the ants were checked for radioactivity (table 1). The ants definitely gathered radioactivity from the beetle, undoubtedly derived from trichome secretions. The next question was whether these radio-active-labelled secretions could be spread to other ants by social food distribution. To determine this, the ant (A) was enclosed for 20 hrs in a small container (surface 1.5 cm^2) with 2 ants of the same colony. During this time no food was added. The results are shown in table 2. The donor ant, which was in direct contact with the radioactive beetle, dispersed radioactive material by social food distribution. The reason that the sum of counts of the donor ant (plus acceptor ants) is higher than the original rate of the donor ant is a result of the technical problem discussed earlier (Kloft, 1962).

Table 2. Transfer of radioactivity, gathered from a radioactive *Cremastocheilus castaneus* (table 1) to further ants via regurgitation within 3 hrs.

	COUNTS	COUNTING DURATION (MIN)	CPM	$\pm$ SQ. RT. MIN
BACKGROUND	56	5	11.2	$\pm$ 3.34
BACKGROUND	48	5	9.6	$\pm$ 3.09
DONOR ANT = ANT A FROM TABLE I	97	5	19.4	$\pm$ 4.40
1. ACCEPTOR ANT	81	5	16.2	$\pm$ 4.02
2. ACCEPTOR ANT	61	5	12.2	$\pm$ 3.49

Detection of further trichome areas on the venter of *Cremastocheilus*

Ever since *Cremastocheilus* has been known to inhabit ant nests, there has been speculation about the glandular nature of several morphological structures. This has usually centered around the large tufts of coarse setae (trichomes) located on the anterior (figs. 1a, 2a) and posterior (figs. 1c, 2c) angles of the pronotum (Hoelldobler, 1971). Other areas that have been previously suggested include the enlarged and projecting propygidial spiracles (figs. 1d, 2d), although there is no evidence for it.

It was therefore quite surprising when the structure located between the anterior coxae (fig. 2b) was noted as attractive to the ants during this study. This
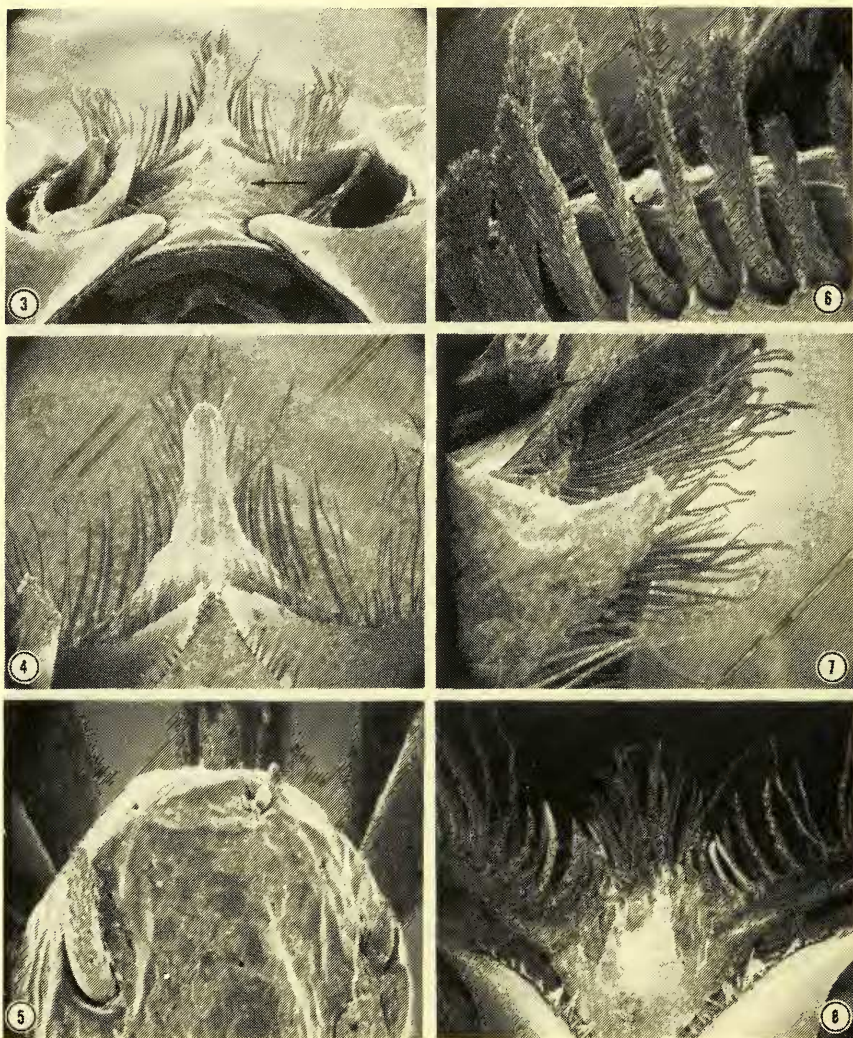
Figs. 3—8. Prosternal apophysis of *C. castaneus brevisetosus* (anterior legs removed): 3) postero-ventral view (45 ×); 4) enlargement of fig. 3 (95 ×); 5) enlarged tip of apophysis (925 ×); 6) sensors on margin behind apophysis, enlargement of area at arrow in fig. 3 (750 ×); 7) lateral view of apophysis (100 ×); 8) ventral or head on view of apophysis (100 ×).

structure (figs. 3—8) has been termed a prosternal apophysis by Krikken (1976). It projects forward toward the notch in the posterior margin of the mentum (fig. 19) and would appear to fit there if the head or mentum were extended down or back. The apophysis is fringed with very long sensors (figs. 3—5, 7—8) which appear to be the trichomes sought by the ants.

During removal of the legs in preparation for the scanning electron microscope studies, an additional group of sensors was located beneath the anterior coxae (figs. 3 arrow, 6). Although previously undetected and well-hidden, the compli-
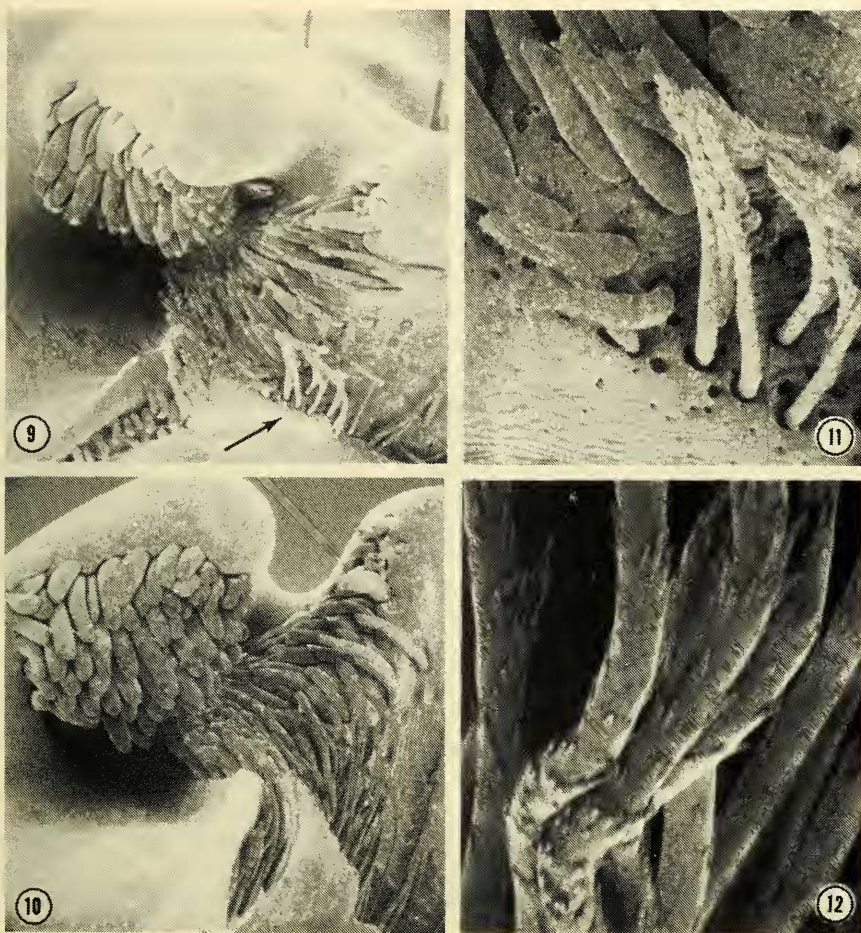
Figs. 9—12. Anterior pronotal trichome area (fig. 1a) of *C. castaneus brevisetosus*: 9) dorsal view of right side (120×), area at arrow enlarged in fig. 11; 10) ditto, angle more dorsal (150×); 11) enlargement of trichomes at arrow in fig. 9 (525×); 12) ditto (1000×).

cated nature of the sensors (fig. 6) suggests that they serve an important function.

Unfortunately the exact behavior of the ant solicitations and the beetle's feeding are nearly impossible to observe carefully. Alpert & Ritcher (1975: 289) stated that "when feeding, a beetle lowered its mentum and pierced an ant larva with its sharp maxillae. The mandibles are greatly reduced and aid in the transport of fluid".

The prominent propygidial spiracles (figs. 1d, 2d) are a possible source of secretions because of their elevated nature and the shape and size of the opening (figs. 17, 18, 20). It is possible that attractant chemicals are produced, although chemicals with a totally different function could be involved. Repellents would certainly be advantageous to a commensal if the host ants became inhospitable and agressive. Alpert & Ritcher (1975: 288) found that "If the beetle was violently disturbed or turned over by the ants, a droplet of viscous fluid was released from

the anal opening. This fluid had an offensive odor and was effective in repelling the ants". They did not mention any observations on the nature of the propygidial spiracles.

The pronotal trichomes are so prominent, and unique to the tribe Cremastocheilini, that their role cannot be overemphasized. The sensors, or individual trichomes, appear to be gnawed upon by the ants (figs. 15—16). Only then is it noticeable that they are hollow (fig. 16). The several shapes of trichomes nearly fill the depressions surrounding them (figs. 1a, 1c, 2a, 2c, 10, 14). The anterior ones (fig. 1a) are accessible to the ants from above; the posterior ones (fig. 1c) primarily accessible from the side; and those on the prosternal apophysis are accessibly only ventrally.
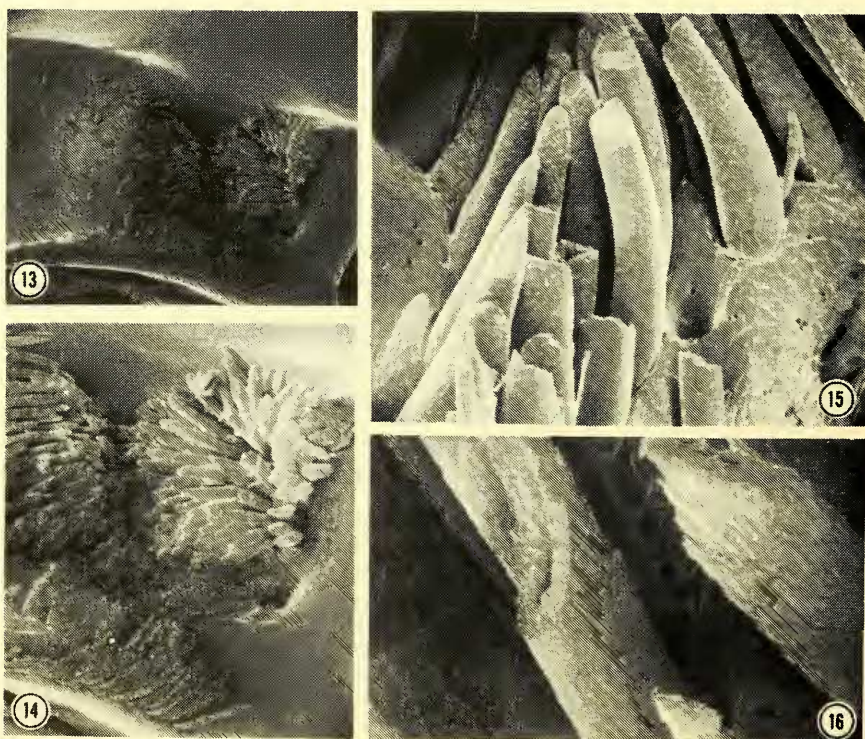
Figs. 13—16. Posterior pronotal trichome area (fig. 1c) of *C. castaneus brevisetosus*: 13) ventral view of left side (55 ×); 14) ditto (105 ×); 15) gnawed trichomes (535 ×); 16) ditto, individual hollow seta of the trichome (1050 ×).

DISCUSSION

According to our results, *Cremastocheilus castaneus* seems to play a double role within the *Formica integra* community. It is first a predator which kills ants, squeezes out juices, and feeds on the contents of the gasters of workers. It also cuts the ants into pieces, possibly feeding on these parts. Since we experimented only with adult workers we couldn't observe feeding on ant larvae, as described in

detail by Cazier & Mortenson (1965) and Alpert & Ritcher (1975). But in spite of being well protected in the sensitive mouth area by the anterior portion of the mentum, which completely covers the mouthparts (fig. 19), the beetles should not simply be regarded as synechtrans. They are, according to Wilson (1971), symphiles (so-called "true" guests). We determined that they are not only groomed but also participated in the social food exchange within the ant colony. The additional trichome areas on the prosternal apophysis help in releasing the food sharing behavior through the ants.

We obtained evidence of the transfer of radioactive material from the beetle to the ants, presumably via trichome secretions. As we expected, an ant which was in contact with a radioactive beetle also distributed the radioactivity, through social food distribution, to other ants which were never in contact with the radioactive-labelled beetles. Thus the "peace-making" allomones (Pasteels, 1977) of the beetle might be distributed to wider parts of the nest population. Possibly during the reproductive activities of the beetles, the production of the attractive and "peacemaking" secretions stop (or may be masked by sex pheromones which are a deterrent for the ants). This would explain the observations of Cazier & Statham (1962) that the beetles are pulled out of the nest and dumped in the refuse zone. In
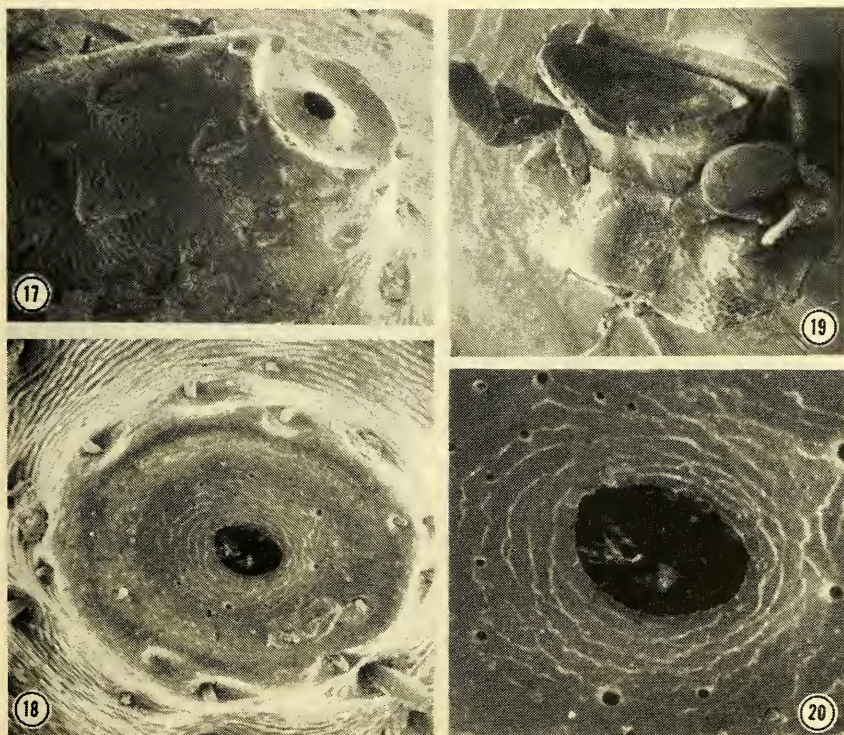
Figs. 17—20. *Cremastocheilus castaneus brevisetosus*: 17) propygidial spiracle, lateral view (210×); 18) ditto, head on view (340×); 19) head, latero-ventral view (26×); 20) enlargement of propygidial spiracular opening of fig. 18 (850×).

the transitional stage, other workers of the same colony might attempt to pull the beetle back in the direction of the nest. This dumping and pulling back lasts a certain time, but finally the beetles fly away. Hoelldobler (1971) showed that myrmecophiles communicate in the same chemical language as their hosts. He defined trichomes as "... tufts of hairs that serve to increase the ability of well-integrated ant guests to communicate chemically. They are located on the ventral surface of highly modified anterior and posterior pronotal projections of *Cremastocheilus* adults". Mating and breeding areas are not yet known, but mating outside the colonies would be advantageous for gene flow.

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